

# Investigation of refractive index dynamics during *in vitro* embryo development using off-axis digital holographic microscopy: supplement

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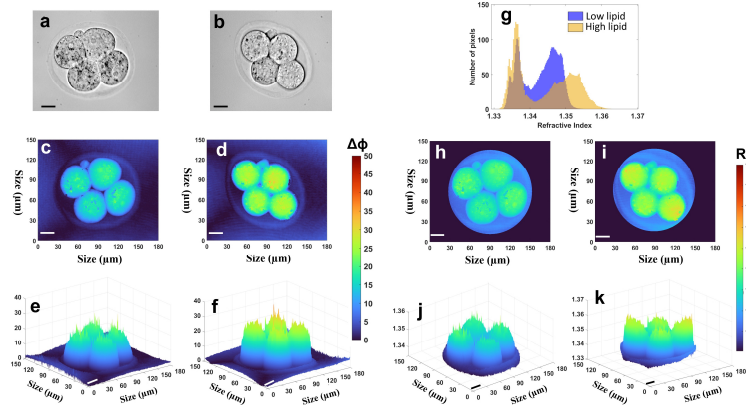
## 1. FIGURES AND TABLES

**Table S1. Sample sizes for the different embryo developmental stages imaged**

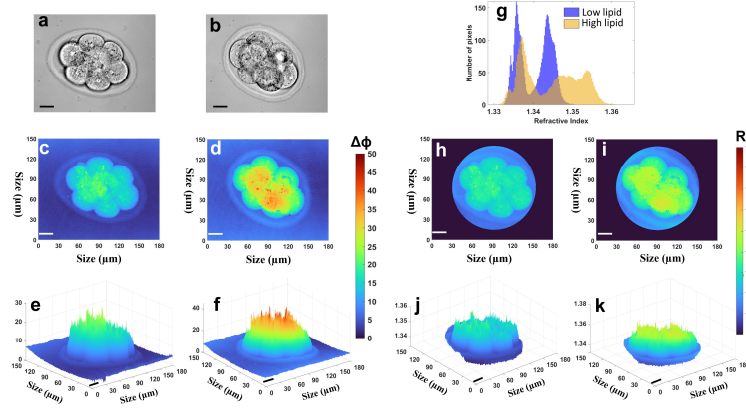
	Low lipid	High lipid
2-Cells	11	14
4-Cells	22	21
8-Cells	22	37
Morula	29	42
Blastocysts	28	15

**Table S2. Average measured refractive indices for each developmental stage**

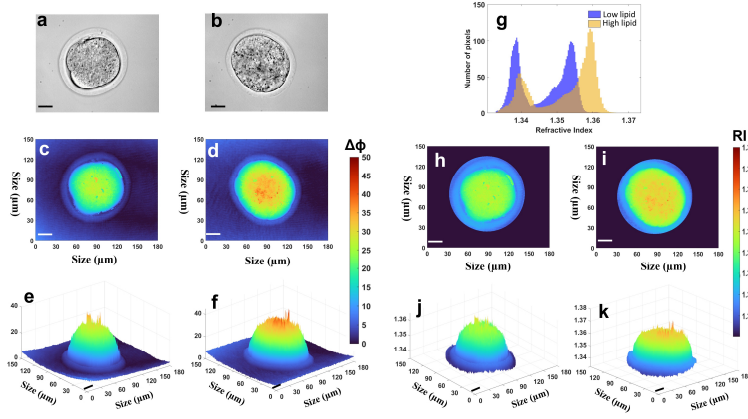
	2-cell	4-cell	8-cell	Morula	Blastocysts
Low lipid	1.347	1.346	1.347	1.346	1.344
High lipid	1.349	1.348	1.349	1.351	1.346



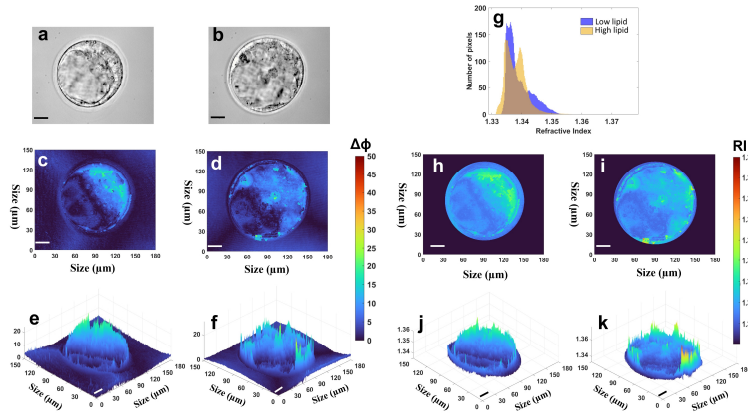
**Fig. S1.** Representative phase information and refractive index maps for 4-cell embryos cultured in low or high lipid media. (a,b) Bright field images for low lipid and high lipid-treated 4-cell embryos, respectively. (c,e and d,f) Reconstructed 2D phase profiles and their corresponding 3D topographical profiles for low lipid and high-lipid group respectively. (h,j and i,k) Reconstructed 2D refractive index profiles for low lipid and high lipid group with their corresponding topographical refractive index profiles. (g) Frequency distribution of all pixels measured using the reconstructed 2D refractive index profiles for low and high lipid groups of embryos in (h and i, respectively). Scale bars = 30  $\mu\text{m}$ .



**Fig. S2.** Representative phase information and refractive index maps for 8-cell embryos cultured in low or high lipid media. (a,b) Bright field images for low lipid and high lipid-treated 8-cell embryos, respectively. (c,e and d,f) Reconstructed 2D phase profiles and their corresponding 3D topographical profiles for low lipid and high-lipid group respectively. (h,j and i,k) Reconstructed 2D refractive index profiles for low lipid and high lipid group with their corresponding topographical refractive index profiles. (g) Frequency distribution of all pixels measured using the reconstructed 2D refractive index profiles for low and high lipid groups of embryos in (h and i, respectively). Scale bars = 30  $\mu\text{m}$ .



**Fig. S3.** Representative phase information and refractive index maps for morula-stage embryos cultured in low or high lipid media. (a,b) Bright field images for low lipid and high lipid-treated morula-stage embryos, respectively. (c,e and d,f) Reconstructed 2D phase profiles and their corresponding 3D topographical profiles for low lipid and high-lipid group respectively. (h,j and i,k) Reconstructed 2D refractive index profiles for low lipid and high lipid group with their corresponding topographical refractive index profiles. (g) Frequency distribution of all pixels measured using the reconstructed 2D refractive index profiles for low and high lipid groups of embryos in (h and i, respectively). Scale bars = 30  $\mu\text{m}$ .



**Fig. S4.** Representative phase information and refractive index maps for blastocyst-stage embryos cultured in low or high lipid media. (a,b) Bright field images for low lipid and high lipid-treated blastocyst-stage embryos, respectively. (c,e and d,f) Reconstructed 2D phase profiles and their corresponding 3D topographical profiles for low lipid and high-lipid group respectively. (h,j and i,k) Reconstructed 2D refractive index profiles for low lipid and high lipid group with their corresponding topographical refractive index profiles. (g) Frequency distribution of all pixels measured using the reconstructed 2D refractive index profiles for low and high lipid groups of embryos in (h and i, respectively). Scale bars = 30  $\mu\text{m}$ .